*end of ChAPTER 4*

**Somatic Hypermutation (SHM)** (Figs. 4.27, 4.28)

# - Occurs only after B cells respond to Ag and have had a chance to proliferate.

- One clone of B cell proliferates to become many identical B cells and they all undergo different mutations in their antibody genes and start expressing variations of the original antibody. Works on V regions (VJ or VDJ segments) not C or other genes (Fig. 4.27).

- requires an enzyme called **AID** (activation-induced cytidine deaminase), that is expressed only in activated B cells. The AID enzyme converts cytosine bases to uracil, which are then converted to other bases by the DNA repair machinery.

*-* The B cells are subsequently selected for their ability to bind the antigen. The B cells that lose specificity to the antigen through mutation will die out (by apoptosis), whereas the ones that bind Ag better will have a selective advantage.

- surviving B cells tend to have mutations affecting amino acid residues of the CDR loops rather than framework regions (Fig. 4.28)

- leads to refinement of Ab specificity (**affinity maturation**) during the course of B cell’s lifetime by mutation of Ig genes followed by selection.

**Antibody Diversity Generation - constant region (heavy chain)**

An antibody can be one of nine different isotypes (IgM, IgD, IgG1-4, IgA1-2, IgE). A given variable region (= a given antibody specificity) can be linked to the constant region of any one of these isotypes.

**Isotype Switching**

- Different isotypes, in order of abundance in serum (Fig. 4.31): IgG (four subclasses: IgG1, IgG2, IgG3, IgG4), IgM, IgA (two subclasses: IgA1 and IgA2), IgD and IgE

- Functional differences: depends on the Fc portion of a given Ab

- Structural differences: number and location of disulfide bonds, number of oligosaccharides, number of C domains, and the length of hinge region (Fig. 4.5).

- How are the different isotypes generated?

- All B cells start by making IgM and IgD: C and C are the closest to the variable region. (Fig. 4.30)

- The same VH genes (already rearranged) can be linked to different CH genes during immune responses – **isotype switching** or class switching. The molecular process by which the isotype is changed is called **class switch recombination (CSR).**

- There are **switch regions** in front of each CH gene for each isotype (except ), and a recombination mechanism allows excision of intervening sequences.

- The DNA in the switch regions is nicked (single strand break) through a process initiated by the enzyme **AID**. Humans lacking AID make only IgM antibodies, of low affinity.

- nicked DNA in Sµ and other switch region leads to recombination

- Sequence of events in Ig isotype switch

- B cells switch their heavy chain constant region genes after an Ag encounter to express IgG, IgA, or IgE (instead of IgM and IgD). Isotype switch (CSR) is prompted by cytokines B cells encounter; usually secreted by T cells. Some stay as IgMs.

- Further isotype switching (of downstream genes) can occur (i.e. IgG1 to IgA) but can't go back to "upstream" isotype. (Fig. 4.30)

Isotype switching allows the same Ag specificity (V region) to be linked to C regions with different effector functions (Fig. 4.32)

-Differences between VDJ gene rearrangement and isotype switching (CSR)

1. Unlike variable region recombination, all recombination for isotype switch results in functional protein products because the switch sequences for isotype switch lie in the intron.

2. Different switch signal and machinery. *Discuss RAG, RSS, etc.* The enzyme activation-induced cytidine deaminase (**AID**) is required for both CSR and SHM.

3. Variable region recombination for both heavy and light chains occurs during B cell differentiation in the bone marrow – no further variable region rearrangement occurs once B cell reaches the periphery (somatic hypermutation occurs in the periphery, but this is not gene rearrangement but instead mutations). Constant region rearrangement (isotype switching) occurs only in the periphery.

4. Type of isotype is not determined randomly - regulated by T cells (cytokines)

Useful to review Fig. 4.35

# Distribution and function of Antibody isotypes (from Chapter 9, second half)

- Most common site of pathogen introduction: respiratory, digestive, and urogenital tract, and damaged skin. Mucosal surface, tissue, and blood all need protective antibodies.

- Different **isotypes** are found at different sites (Fig. 9.23).

- The same antigen specificity (VH and VL) can be linked to different isotypes by class switching; thus a single B cell can produce antibodies, all specific for the same antigen, that provide all the protective functions appropriate for each body compartment.

- Each isotype has a different size, distribution, and function (Fig. 4.32).

- IgM: - First Ig to be expressed on B cells, first isotype secreted in immune response.

- Tend to be lower in affinity. Makes up for it by being a pentamer when secreted. (Fig. 4.29)

- held together by disulfide bonds with **J chain**. *don't confuse with J gene segments.* Enhances **avidity** (affinity x number of binding sites), important for IgM that is produced before somatic hypermutation. IgG etc. have higher affinity because SHM and CSR occur concurrently in activated B cells.

- IgM found in the blood (large size prevents it from crossing into tissues except during inflammation); together with IgG the IgM in the blood is important to prevent blood-borne infection

- Do not have Fc receptors so do not opsonize directly

- However, potent activator of complement cascade leading to opsonization (see below)

- IgMs are produced even during secondary and subsequent immune responses and after somatic hypermutation, but other isotypes dominate.

- IgG: - Principal isotype in the extracellular fluid. IgG is small enough to diffuse out of the blood vessels into tissues, and is also actively transported by a Fc receptor called **FcRB** (Fig. 9.21; also known as FcRn as shown in this textbook)

- Maternal-fetal transfer directly through placenta in utero, again via FcRB.

- IgG is effective in complement fixation and opsonization (esp. IgG1 and IgG3). (Fig. 4.32)

- IgG is usually high affinity and can neutralize bacterial toxins.

- IgA: - Principal type in secretion to body cavities.

- Weak opsonin and a poor complement activator - mostly neutralizing.

- IgA-secreting plasma cells are found in **lamina propria**, just under the basement membrane of many surface epithelia.

- IgA is secreted as a dimer joined by J chain (same as for pentameric IgM). (Fig. 4.33)

- Major areas of IgA synthesis: gut, respiratory epithelium, lactating breast, tear and salivary glands.

- Breast milk contains IgA and protects an infant's gut from infection. (Fig. 9.23)

- IgA in milk and IgG transfer across placenta are examples of **passive transfer of immunity**.

- dimeric IgA is transported from lamina propria to the surface of epithelium by **poly-Ig receptor** via **transcytosis** (Fig. 9.22).

- Once on the apical surface poly-Ig receptor is enzymatically cleaved and IgA is secreted with a part of the poly-Ig receptor still attached (called **secretory component**)

- IgA neutralizes bacterial toxins and can prevent attachment.

- monomeric IgA can also be made, mainly by plasma cells in lymph nodes and spleen, and can neutralize viruses and toxins in blood and tissues

Antibody production in newborns (Fig. 9.24)

 - between 3-12months babies are most susceptible to infection because maternal IgG is lost from the circulation, IgA (milk) from mother is reduced and their adaptive immune system is not yet mature

- IgE: return to this isotype in allergy discussion in next lecture

### Effector functions

## Neutralization

Mostly carried out by IgG and IgA, abundant in extracellular spaces and body cavities, respectively.

1. Virus

- Viral infection can be blocked by neutralizing antibodies.

- i.e. influenza and **hemagglutinin** (HA) --binds to certain carbohydrates expressed on epithelial cells of respiratory tract. (Fig. 9.25; recommend reading the figure caption)

2. Bacterial attachment

- Some bacteria need to attach to epithelial cells (i.e. Gonorrhea) or extracellular matrix (e.g. Strep) in order to infect.

- IgA can bind and neutralize attachment. (Fig. 9.26)

3. Toxins

- Many toxins harm by mimicking cellular counterparts

- Many toxins can harm in very small quantities: important for the Ab to diffuse into the tissue fast, bind toxins rapidly and with high affinity.

- Neutralization prevents toxin attachment to host cells (Fig. 9.28)

- Immunization: diphtheria and tetanus toxins are denatured (now called **toxoid**) and given to infants. Toxoids lack toxic activity but retain the antigenic epitopes and therefore induce an immune response to the toxoid and the native toxin.

- **Passive immunization**: when there is no time to induce adaptive immunity (snake venom), neutralizing Ab from another organism is injected (horse).